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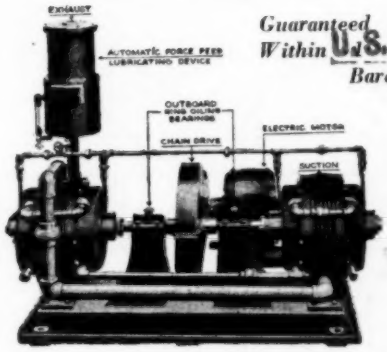

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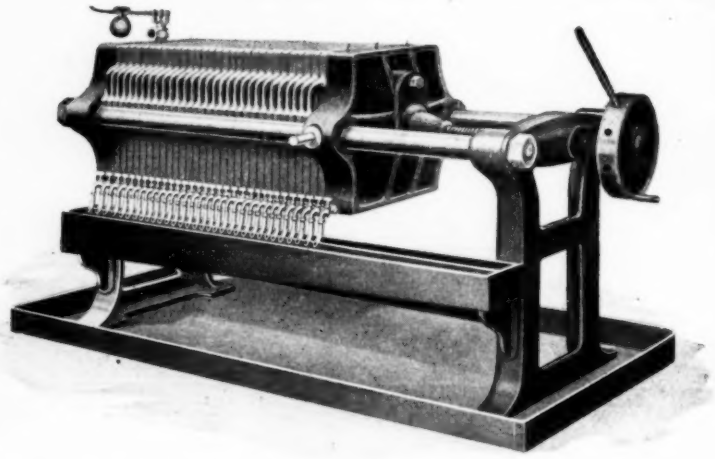

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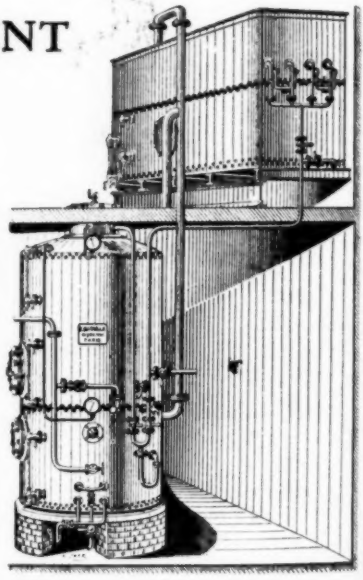

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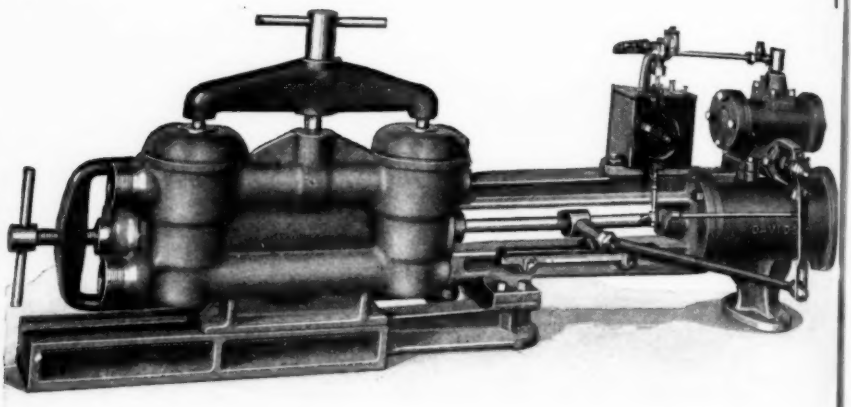

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VOLUME V APRIL, 1928 NUMBER 4

Deflocculation and Detergency Not Entirely Correlative

A Report on Detergent Experiments on Cotton Soiled with Carbon Black

BY ROBERT M. CHAPIN*

AFTER work presented by the writer[1] on the deflocculation of carbon black, it seemed desirable to perform detergent experiments upon fabrics soiled by the same substance with the object of determining whether deflocculation and detergency are correlative phenomena.

The Washing Machine:—Comparative washing tests should be conducted under uniform conditions of agitation and "rubbing," and so practically demand the use of some sort of machine. Whether a laboratory machine imitates any commercial washer in action seems of slight importance, for the determination of the relative efficiencies of various machines is essentially an independent problem. The machine used was devised to wash and rinse small slips of fabric with vertical agitation in test tubes immersed in a water bath. The slips were held at one end in spring clips suspended from rocker-arms and were weighted at the free ends. Construction drawings are given in Figures 1 and 2. The machine carried four double rocker-arms accommodating eight clips with their suspending shafts. The shafts were equipped with perforated metal discs to cover the test tubes. The supporting frame was made of steel, painted; the pulley of wood; the rest of the apparatus of hard brass.

The motive power was a 1/50 hp. motor equipped with a variable friction gear and further controlled by a slide rheostat. The belt, on its traction side, ran over a small pulley attached to the shaft of a bell revolution counter, so that the speed of the machine, always kept close to 300 r.p.m., could be audibly checked.

The bath was an ordinary enamelled pail, about 11 inches in diameter at the top. On this was fitted a cover of sheet brass, containing

[1] Ind. & Eng. Chem. *18* 1313 (1926); *19* 1275 (1927). O. & F. Ind. *4* 15,210 (1927).

*Biochemic Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.

two rows of holes to accommodate the test tubes, the rows being spaced 3⅞ inches between centers. A hole for a thermometer was also provided. The test tubes were of Pyrex glass, 25 x 200 mm. Each was equipped with a tightly fitting ring of wire drawn through small rubber tubing, the lower edge of the ring being set 6 inches above the inside of the bottom. These rings supported the tubes in position in the bath. When set up the apparatus was adjusted to afford clearances of 1¼ inches between the extremities of the clips at their lowest points of travel and the bottoms of the test tubes.

Preparation of the Fabric:—New fabric, in 18-inch squares, was boiled gently for 1 hour in a liberal volume of a 0.5 to 1.0 per cent solution of neutral, silica-free white soap in a beaker provided with a grating of nichrome gauze to keep the goods off the bottom. After cooling to 40°-50° C. the goods were plunged by hand about 2 minutes, then squeezed strongly and transferred to a similar beaker in which they were simmered 15 minutes in a 1 per cent solution of concentrated ammonia water. After again cooling, plunging and squeezing they were given a second, and then a third similar rinsing with diluted ammonia. During the washing and rinsing the goods were frequently overhauled and punched with a stout glass rod. From the last rinse they were removed dripping wet, dried on clean sheet

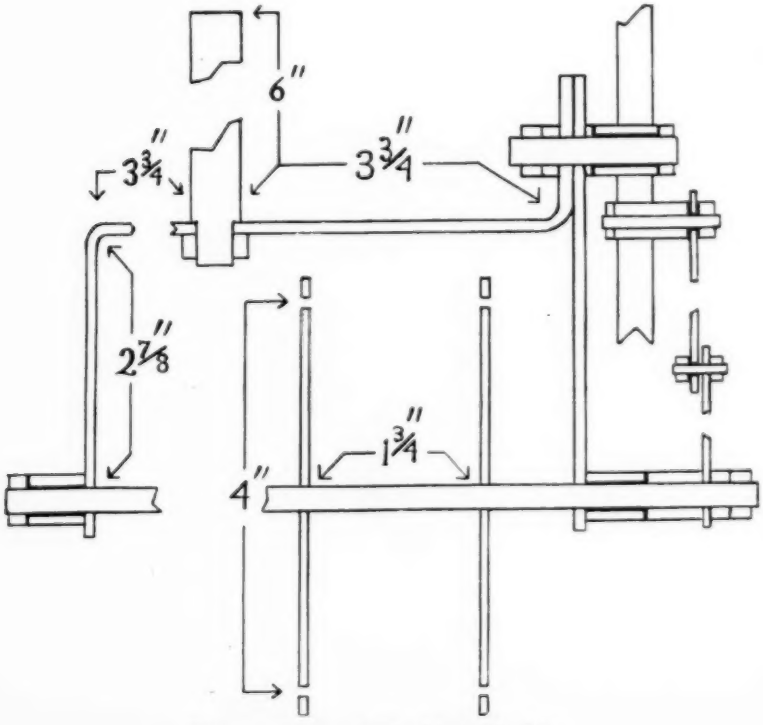

Fig. 1.—Plan of Washing Machine.

glass, and cut into slips 2 x ¾ inches for heavy goods or 2 x ⅞ inches for light goods, the longer dimension being parallel to the selvage. Some were then used without further preparation; others were first extracted with ether in a Soxhlet apparatus. All were provided with lead weights before use. Sheet lead, about 1/16 inch thick, was cut into ⅜-inch squares, the pieces averaging about 1.7 g. each. A square was bent into a V with pliers and pinched firmly onto an end of each slip.

To insert a slip into a clip the latter, with its jaws held uppermost, was slipped over the handle of a metal spatula, horizontally clamped to a ring stand. A slight movement of the shaft then sufficed to pry the jaws of the clip sufficiently open to allow the unweighted end of a slip to be evenly inserted for about ⅛ inch.

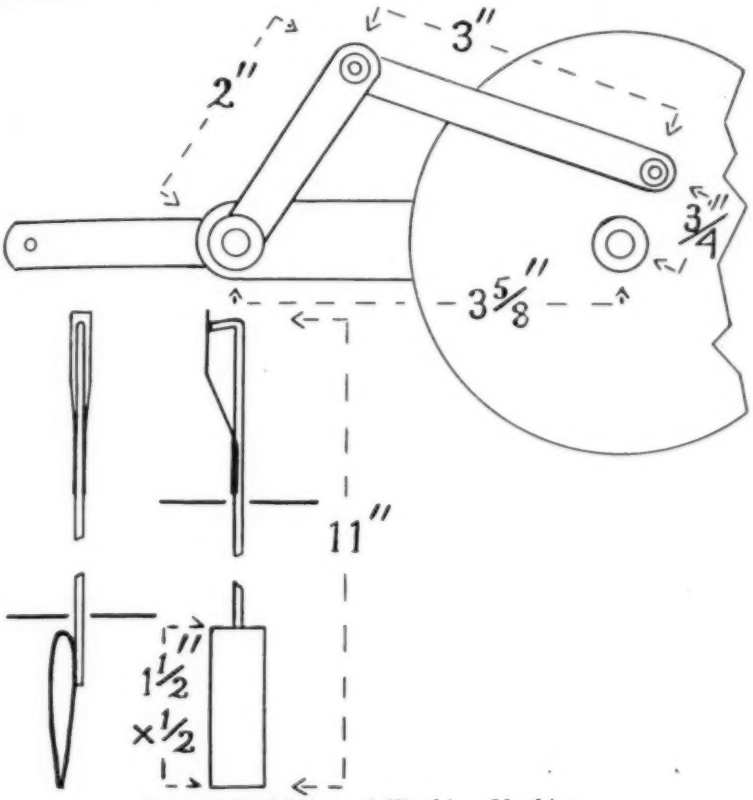

Fig. 2.—End View of Washing Machine.

Evaluation of the Residual Soil:—In order to arrive at a numerical expression for the soil remaining on a piece of washed fabric, a colorimeter was converted into a reflectometer in a manner which the writer has not seen described in the literature. The two mirrors of a Kober-Klett instrument were replaced with a single rectangle of plate glass smoothly covered with dull black woolen cloth. This will be termed the "reflecting table" of

the instrument. Only uniform artificial illumination was employed. First, a slip of the unsoiled fabric was smoothly laid down at each end of the reflecting table so that its half-field in the eyepiece was completely filled. Second, the two cups were charged with a slightly acid and perfectly clear solution containing cobalt, nickel and copper salts in appropriate proportions, and both plungers were set at the same reading, say 30 mm. Third, the instrument was shifted sideways in front of the lamphouse until the half-fields in the eyepiece exactly matched. Fourth, one of the cups, say the right-hand, was emptied, rinsed and charged with distilled water, and the right-hand slip was removed from the reflecting table. The apparatus was then ready for use. A slip carrying soil to be evaluated was laid on the right-hand end of the reflecting table and the left-hand plunger was moved until the half-fields matched, the right-hand plunger being also moved nearly in unison. After the reading of the left-hand plunger had been taken the soiled slip wa turned over and the process was repeated. The readings obtained against the two sides of the soiled slip were averaged for the final result.

The appropriate blending and dilution of the standard solution of cobalt, nickel and copper salts had to be determined by repeated trials, and probably would vary with different light-sources. The numerical result for residual soil was therefore directly obtained as the millimeters read on a particular colorimeter against a standard that was of unknown, though probably of permanent, composition. If it should prove worth while, such results could be recalculated so as to make the detergent power of some standard soap solution the ultimate unit for comparison.

Cleansing from Initially Adsorbed Carbon Black

In the first attack upon the problem it was desired to keep the composition of the system as simple as possible. Therefore the slips were soiled by agitation in a mixture of carbon black and water only. The carbon black was the identical lot used in the previously published experiments on deflocculation. It seemed to be strongly adsorbed by the fabric but naturally only upon the surface, being unable, in its coarsely suspended condition, to penetrate deeply into the threads.

The soiling was done at room temperature. Test tubes were charged with 0.3 gram carbon black and 30 cc. distilled water, stoppered, given a brief vigorous shaking, and placed in position in the machine. Slips of weighted fabric in their clips were introduced and the machine was run 8 minutes. Some slips were then given a 2-minute rinse in 30 cc. distilled water; others had received in addition a preliminary 2-minute rinse before entering the carbon black suspension. All were finally laid on glass plates, dried at 100° C. and stored in paper boxes.

Experiment 1.—The first experiment dealt with the effect of duration of washing. Tubes were charged with 30 cc. of 0.03 N pure, neutral, sodium palmitate, heated to incipient boiling, stoppered and held in the bath at 70° C. for 1 hour. Then soiled slips were introduced, washed varying periods, rinsed 4 minutes in 50 cc. water at 70° C. and dried on glass. In Figure 3, Curve I, are shown the

results on a light-weight plain nainsook, not ether-extracted, soiled without either a preliminary or final rinse. Curve II shows the results on a light handkerchief linen,

Experiment 4.—To observe the effect of temperature, tests were run on neutral sodium stearate as in Experiment 2 except that the temperature was 60° C. The re-

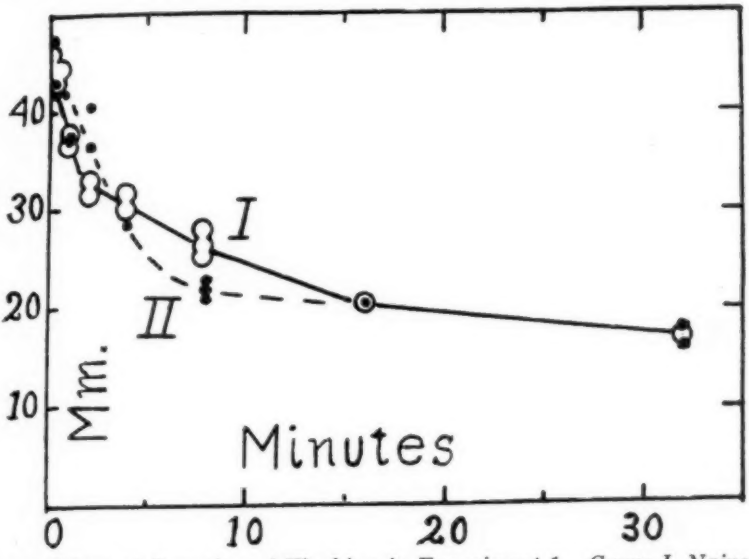

Fig. 3.—Effect of Duration of Washing in Experiment 1. *Curve I, Nainsook; Curve II, Linen.*

which had been ether-extracted and also given the double rinse when soiled.

Experiment 2.—Sodium stearate, neutral, acid and alkaline, was tested at 70° C. upon nainsook slips, ether-extracted and soiled with the double rinse. The soap solutions were all held 1 hour at 70° C., after incipient boiling and thorough mixing, before the goods were introduced. The wash period was 8 minutes; the rinse 4 minutes. The results are given in Figures 4 and 5.

Experiment 3.—Following Experiment 2, it seemed necessary to test the effect of sodium hydroxide at 70° C. in absence of soap, with the results shown in Curve I of Figure 7.

sults are shown in Curve I of Figure 6. Above 0.04 Normal the soap solutions were too thick to be workable. Results at 25° C. are given in Curve II, Figure 6.

Experiment 5.—Neutral potassium stearate, tested at 25° C. but otherwise according to the technique of Experiment 2, gave the results shown in Curve II of Figure 7.

Experiment 6.—Neutral sodium laurate was tested at 25° C. and at 70° C., but otherwise according to the technique of Experiment 2 with the results shown in Figure 8.

Experiment 7.—Inasmuch as some of the experiments on deflocculation had indicated that sodium laurate might show notably

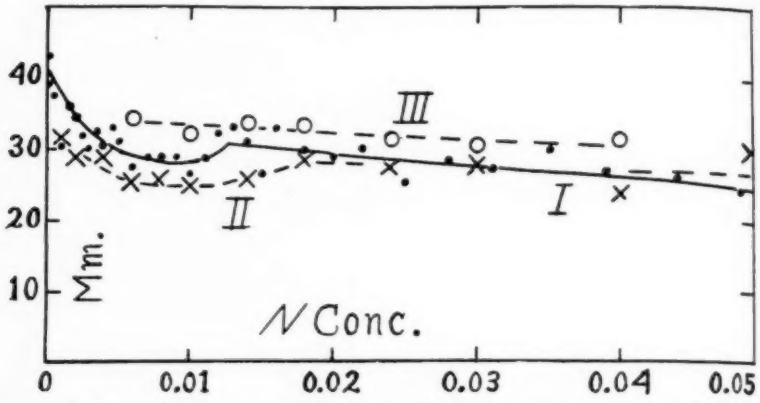

Fig. 4.—Sodium Stearate at 70° C. in Experiment 2. Curve I, Neutral; Curve II. Alkaline; Curve III, Acid.

increased power when crystals of the substance were present, tests were run at 25° C. in which the standard temperature was approached from below. The tubes of prepared soap solution were chilled 1 hour in ice water, then held 1 hour in the bath at 25° C., being stirred with a glass rod toward the end of the period, after which the experiment was completed according to the technique of Experiment 2. Crystals were abundantly present at the time of washing at concentrations of 0.20 and 0.16 Normal, but the results practically coincided with the corresponding results in Experiment 6. They also coincided at concentrations of 0.12 and 0.08 Normal, in which crystals were absent but at 0.06, 0.04 and 0.02 Normal they indicated a loss in detergent power.

Experiment 8.—Neutral sodium oleate from Oleic Acid U.S.P. was tested according to the technique of Experiment 2. In Figure 9 the results at 25° C. are shown as dots, those at 70° C. as circles.

Experiment 9.—A few tests were run on substances other than soap for comparison with data on deflocculation previously secured. The test slips were of ether-extracted

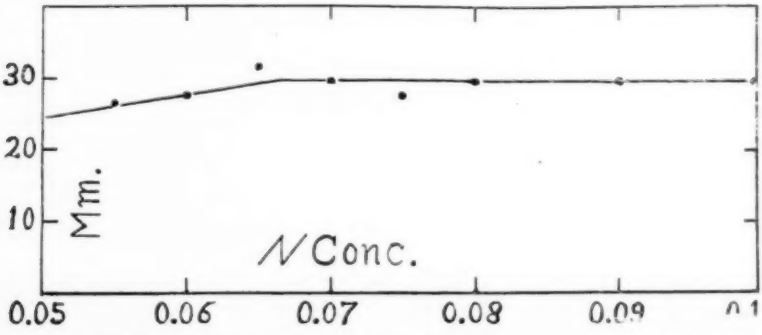

Fig. 5.—Extended Curve of Neutral Sodium Stearate at 70° C. in Experiment 2.

nainsook soiled with the double rinse. The temperature was 27°-28° C. All solutions were left in the bath 1 hour before test. The wash-period was 8 minutes, with a 4-minute rinse. The data are given in Table 1.

Experiment 10.—Similar soiled slips of nainsook were soaked for about 1 hour at room temperature in various liquids, drained briefly and then washed in 0.03 Normal neutral sodium palmitate at 70° C. for 8 minutes with a 4-minute rinse. Preliminary experiments had revealed no difference between sodium palmitate and stearate at this concentration and temperature. The data are given in Table 2.

Experiment 11.—A slip of similarly soiled nainsook from which the lead weight had been removed was sewed by the two ends to the inside of a pair of white duck pants worn in the laboratory, being placed at the back, slightly below the waist-band, and the soiled pants were sent to the laundry as usual. On return the strip was cut off, wet out in hot water and dried on a glass plate to smooth it. It then read at 17 mm. in the colorimeter. A similar experiment at a later date afforded a reading of 13 mm.

Discussion.—The method is decidedly less sensitive to variations in the nature and proportion of soap, and to other influencing factors, than is the previously described method for testing deflocculating power. It is also lacking in precision, largely owing, no doubt, to the initial non-uniformity of the test slips soiled in the manner described. As it stands, it is not a practical routine method for determining the relative detergent powers of competing commercial soaps. However, the results do seem to support the following generalizations respecting the washing of cotton, and probably linen, goods soiled by adsorbed non-oily carbon black:—

(a) There is an optimum temperature for each soap; sodium

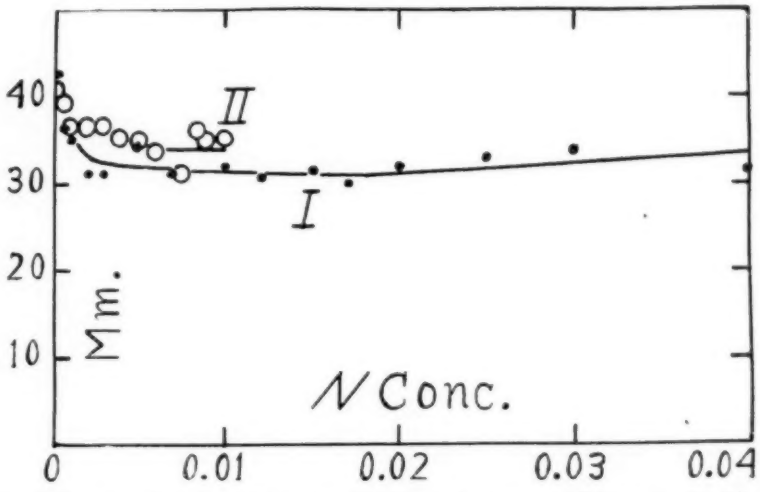

Fig. 6.—Neutral Sodium Stearate in Experiment 4. Curve I, at 60° C.; Curve II, at 25° C.

laurate was more powerful at 25° C. than at 70° C., while the reverse was true of sodium stearate. An oleate appears the best soap for use with cold water, while at or above 70° C. a stearate or palmitate appears more powerful.

(b) Alkali, in moderate concentration, appears to possess slight detergent power by itself and it may decidedly enhance the power of soap solutions. Power is diminished by excess of fatty acid.

(c) Some other substances showed significant detergent power but none was found equal to soap at its best.

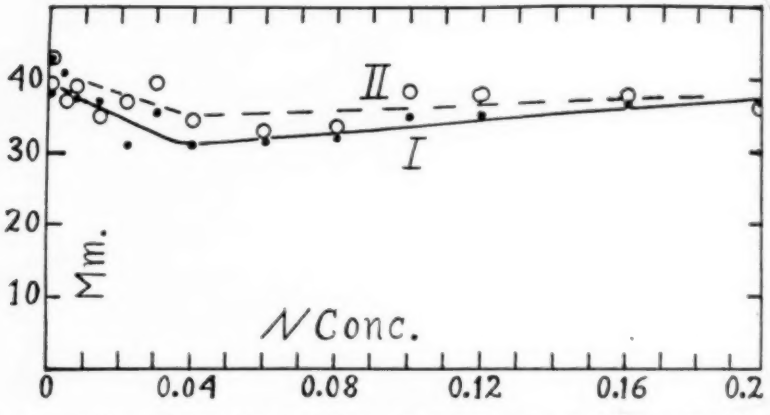

Fig. 7.—Sodium Hydroxide at 70° C. in Experiment 3 (Curve I); Neutral Postassium Stearate at 25° C. in Experiment 5 (Curve II).

(d) No addition other than alkali was found to enhance the detergent power of soap.

(e) Detergency and deflocculation were found parallel in the fact that excess alkali enhanced power while excess fatty acid decreased it, but other possible resemblances were vaguely defined at best.

In the course of the work it was observed that the violence of the "rubbing" may considerably affect apparent detergent power. Occasionally ravellings would become entangled across the face of a strip and the friction would produce a pale streak. Also some experiments were made with slips of extra heavy cotton shirting and it was found that the goods were so stiff that the flexions during washing were likely to occur mostly across the middle with consequent production of a transverse pale band.

Prevention of Adsorption of Carbon Black

Inasmuch as the experiments on the removal of initially adsorbed carbon black had been unsatisfactory, the problem was approached from the other direction. It seemed possible that a comparison of the powers of soap solutions to prevent adsorption of carbon black by a fabric might afford a more sensitive and precise method for practical application.

The fabric employed was "Burton's Irish Poplin," a mercerized cotton, washed, ether-extracted, cut into slips and weighted as before described. This material was easier to handle and seemed to work

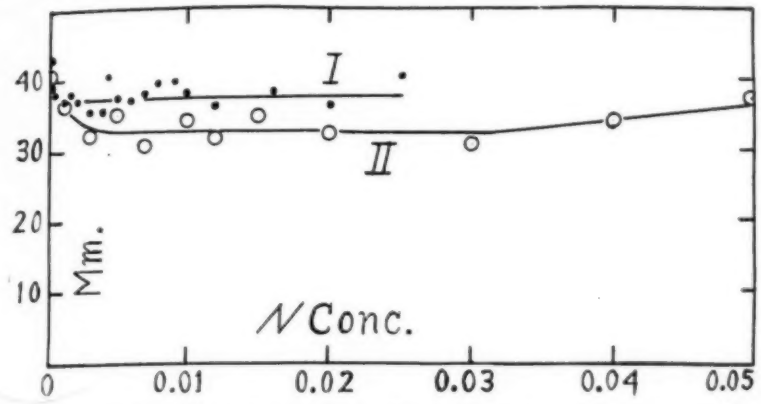

Fig. 8.—Neutral Sodium Laurate in Experiment 6. Curve I, at 25° C.; Curve II, at 70° C.

more uniformly than nainsook. First, the test tubes were charged with 30 cc. soap solution, heated with mixing to incipient boiling, stoppered and left in the bath at the desired temperature for 50 minutes. Second, charges of 0.3 g. of the same carbon black as previously used, already weighed out on celluloid slips, were brushed into the tubes and clips were inserted each of which carried a loop of small brass chain made from a 6-inch length with its ends joined. The machine was now run for 8 minutes. Third, the clips carrying the chains were removed and other clips carrying clean slips were inserted. The machine was now run 8 minutes. Lastly, the slips were given a 4-minute rinse and the residual soil was evaluated as in the previous experiments.

Experiment 12.—The effect of temperature was tried on neutral pure sodium palmitate with the results down in Figure 10. Also the curve at 40° C. was coincident with that at 55° C. so far as it could be carried without undue

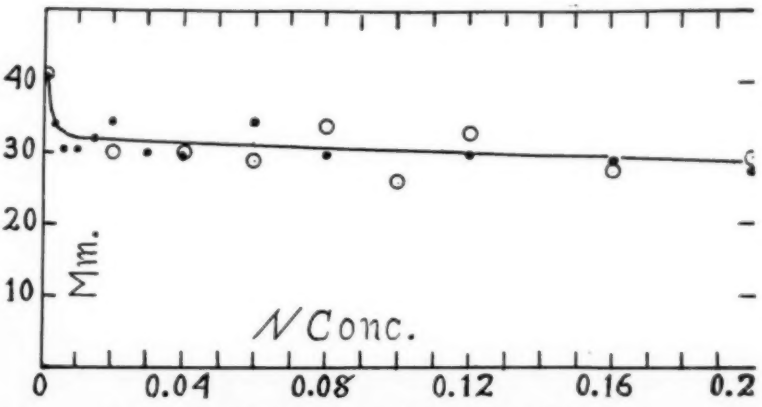

Fig. 9.—Neutral Sodium Oleate in Experiment 8.

thickening of the solution, namely, to 0.01 Normal.

Experiment 13.—Results with neutral sodium laurate at 25° C. are given in Figure 11. The

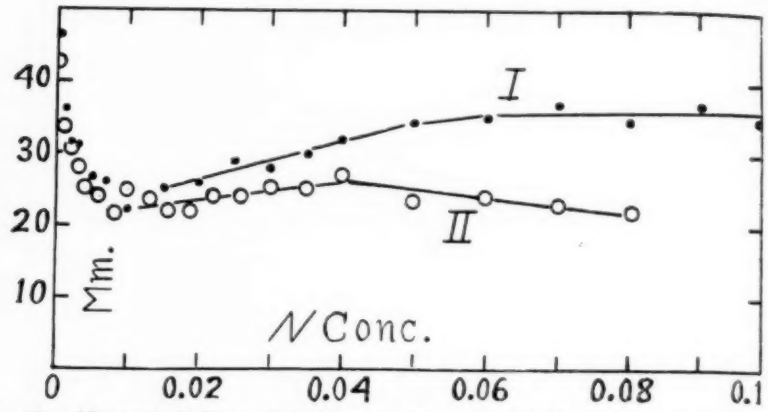

Fig. 10.—*Neutral Sodium Palmitate in Experiment* 12. *Curve I, at* 70° *C.; Curve II, at* 55° *C.*

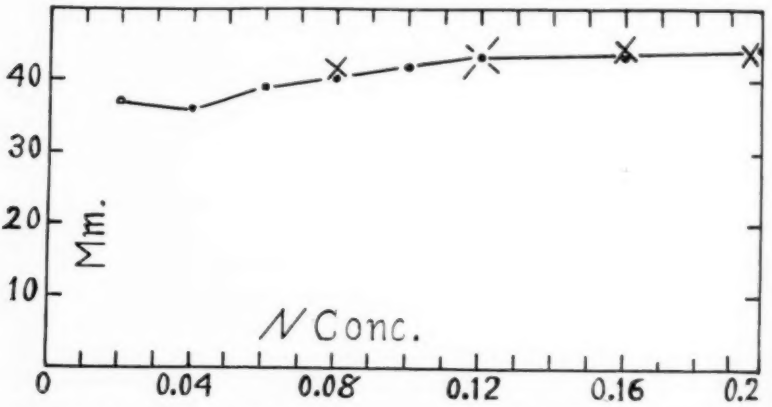

Fig. 11.—*Neutral Sodium Laurate at* 25° *C. in Experiment* 13.

crosses indicate other tests in which the final temperature was approached from below, as in Experiment 7.

Experiment 14.—Acid and alkaline sodium stearate was tested at 70° C. with the results shown in Figure 12.

Discussion.—Soap clearly inhibited adsorption of suspended carbon black by the fabric; stearates and palmitates more powerfully than laurate. But sodium stearate was a more powerful inhibitor in presence of excess fatty acid than in presence of excess alkali, while in the straight detergent experiments the reverse was the case. The most plausible explanation seems to be that the stearic acid is directly adsorbed by the carbon black as an oily coating and thus prevents the ad-

sorptive affinity between black and cotton from coming into full play. On the other hand the detergent experiments seemed to show that neither stearic acid nor other oily substances possessed significant power to dislodge carbon black, once it had been adsorbed by the fabric. Such being the case it is evident that the power to prevent adsorption of carbon black by clean fabric cannot be regarded as a criterion of the power of the soap as a detergent against the same

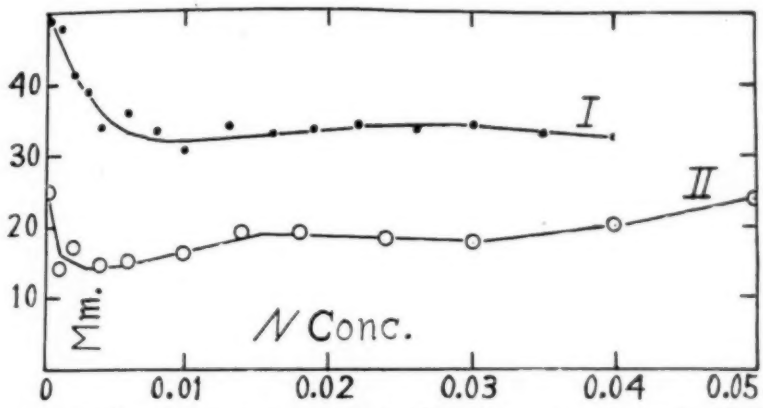

Fig. 12.—Sodium Stearate at 70° C. in Experiment 14. Curve I, Alkaline; Curve II, Acid.

black after adsorption upon the fabric.

These detergent experiments were carried on against adsorbed carbon black for the obvious reasons mentioned in the introduction. But it is doubtful how much they signify from a practical point of view. Fabric in actual service probably seldom becomes soiled with adsorbed non-oily carbon black. It is more likely that such "soot" as may be present carries a distinctly oily film, and accordingly may respond to various detergents in quite a different manner. At all events detergent experiments on fabric soiled with oily carbon black are very desirable.

Summary

1. There is described (a) a laboratory washing machine, and (b) the conversion of a colorimeter into a reflectometer for the evaluation of soil on a fabric.

2. Detergent experiments were performed on cotton fabric soiled in simple aqueous suspensions of non-oily carbon black. The most powerful detergent appeared to be an alkaline solution of sodium stearate or palmitate at 70° C. or above. In this effect of alkali deflocculation and detergency are parallel but other possible correlations were but vaguely defined. The method as given will not suffice for selection of the superior among competing commercial soaps.

3. Comparative tests were made on the power of soap solutions to prevent adsorption of suspended carbon black by clean fabric. Acid soaps were the more effective, probably owing to masking of adsorptive affinities by an oily film of fatty acid. Power to prevent ad-

sorption therefore is not correlative with detergent power in the special case of the prevention of absorption of suspended carbon by fabric. This result is based on tests using soap solutions.

TABLE 1. DATA OF EXPERIMENT 9.

Washing Solution	Colorimeter reading[1]
(a) Hydrochloric Acid; 0.1 Normal	40
(b) Saponin, "Purified," 2 per cent in distilled water, dissolved at room temperature	34
(c) Acacia, 5 per cent, in 0.1 Normal HCl. Acacia was dissolved in water at 60°-70° C., cooled and made to volume with addition of HCl.	33
(d) Casein, "Technical," 3 per cent, in 0.05 Normal HCl, dissolved at 60°-70° C.	31
(e) Gelatine, edible, 5 per cent, in 0.1 Normal HCl. Gelatine was dissolved in water at 60°-70° C., cooled and made to volume with addition of HCl	29

[1] Average of duplicate slips.

TABLE 2. DATA OF EXPERIMENT 10.

Soaking Solution	Colorimeter reading[1]
(a) Neat's-foot Oil	45
(b) Kerosene	34
(c) Cyclo hexanol	33
(d) Sodium Silicate, containing 1.76 Normal Na_2O by titration	31
(e) Cresol, U.S.P.	31
(f) Sodium Hydroxide; Normal	29
(g) Aniline	29

[1] On a single slip only.

OIL CHEMISTS

Make your hotel reservations early for the A. O. C. S. Convention

New Orleans, La., May 14 and 15, 1928

Rancidity Determinations

Calling Attention to a Possible Source of Error in the Kreis Test

BY WILMER C. POWICK*

IT has recently come to the writer's attention that in the examination of fats and oils for rancidity by means of the Kreis test with hydrochloric acid and phloroglucine, very misleading and puzzling results may be obtained through the use of hydrochloric acid containing nitrosyl chloride. While most acids examined, technical and c.p. alike, have been found to be satisfactory for the purpose, the technical acid prepared by one well-known manufacturer has been found to give the well-known ring test for nitric acid and an intense red color with phloroglucine, reacting in both instances in the same manner as pure hydrochloric acid to which a small amount of nitric acid has been added. It is well known, of course, that nitric acid in presence of concentrated hydrochloric acid passes to the form of nitrosyl chloride.

In spite of the strong "blank" test which this acid gave with phloroglucine, negative results were almost always obtained by its use in the Kreis test, even when the fats tested were known to be rancid. Evidently, while the acid was being shaken with the fat prior to the addition of phloroglucine, the nitrosyl chloride was in most cases completely removed by being added across the double bonds of the unsaturated fatty acids and was thus rendered unavailable for reaction with phloroglucine, but in case of rancid fats the removal of nitrosyl chloride was not complete until this compound had first destroyed the epihydrine aldehyde radical to which a truly positive Kreis test is due. That nitrosyl chloride is added across the double bond of oleic acid with the formation of the nitroso-chloride of elaidic acid, has been shown by Tilden and Forster (J. Chem. Soc. London, 65, 329); while direct experiment has shown that the epihydrine aldehyde radical in epihydrine aldehyde diethyl acetal is rapidly destroyed by hydrochloric acid containing nitrosyl chloride. In case of several fats with comparatively low iodine numbers, the reaction between the nitrosyl chloride and the unsaturated fatty acids appeared to proceed, as would be expected, more slowly, so that nitrosyl chloride was still present when the phloroglucine was added, as evidenced by the production of a red color of lower intensity than that obtained from the test on the acid alone. Spectroscopic examination of this color, naturally failed to show the absorption band centred at wave length 5400 A°, which is characteristic of the color similarly obtained from rancid fats by use of pure acid. Furthermore, even in case of these fats with comparatively low iodine number, the nitrosyl chloride could be removed from the impure acid by shaking the fat and acid together for a sufficient length of time—in

*Biochemic Division, Bureau of Animal Industry, U. S. Department of Agriculture.

some cases as much as ten minutes were required,—after which color formation was entirely lacking upon addition of phloroglucine.

It thus appears that no valid results can be obtained in the Kreis test if the acid, because of the presence of nitrosyl chloride, gives a "blank" test with phloroglucine. Under these conditions, rancid fats will generally appear to be sweet, while sweet fats may occasionally appear to be rancid unless the positive reactions be spectroscopically controlled. An illustration of the confusion that may result from this source was kindly brought to the author's attention by Mr. A. W. Putland, of Portsmouth, Va., who, as evidenced by Kreis tests performed with the acid in question, was consistently obtaining an apparently rancid hydrogenated product from apparently sweet non-hydrogenated cottonseed oils. Actually, the hydrogenated products examined by the writer showed no trace of rancidity when the Kreis test was performed with pure acid.

While the Kreis test affords a reliable means for detecting rancidity, or incipient rancidity in fats and oils, it must of course be used with circumspection. In the absence of spectroscopic control, as has elsewhere* been pointed out, positive tests obtained from cottonseed oils are not sufficient evidence of rancidity. It now develops that the test may be completely invalidated, in case of all fats and oils, positive and negative tests alike, by the use of an unsatisfactory reagent.

* Powick: Compounds Developed in Rancid Fats—Jour. Agric. Research Vol. XXVI., No. 8, pp. 336-338, November 24, 1923.

A Useful Addition to Laboratory Extraction Technique

BY PAUL L. MENAUL

The laboratory technique in the determination of oil in cottonseed meal samples appears so simple that no "stunt" could be added to insure greater precision. Yet a brief survey of the reports of the A. O. C. S. Meal Samples discloses a too wide variation in the reports of the oil content; a variation of 0.35% to 0.85% between extremes.

To the undersigned the best technique seemed to be to enfold the sample in a 12.5 cm filter paper, which is then enrolled in another 12.5 cm filter paper, placed in the extraction tube and extracted the required time. However carefully this is done, an appreciable amount of meal dust appears in the extract, varying with the fineness of the meal and the quality of the filter paper. It is rarely that a water clear extract can be obtained.

The author is using a most simple stunt in connection with this method, which invariably yields water clear extracts, and complete extraction of the oil.

This stunt is to moisten the mere lower tip of the outside paper with distilled water. The papers are so rolled that the outer filter paper extends about one half inch below the inner one containing the sample. This empty tip is dipped quickly in distilled water so that only the lower one quarter or one half inch is moistened. *The meal sample must not be moistened!* This moistened tip retains all the meal dust yet does not interfere with the oil extraction. It is also noteworthy that the cheapest filter papers serve as well as the best.

The Status of the Cottonseed Meal Injury Problem*

BY J. O. HALVERSON †

THE author proposes to give briefly the status of the problem involved in the feeding of cottonseed meal, a product which is of great economic importance to the country.

Feeders have long been aware that the *seed* of the cotton plant, unlike that of the wheat plant, could not be fed in large amounts with impunity. Later when the meal was fed in the larger amounts, trouble was encountered also.

Finally, in 1915 Withers and Carruth[1] announced the rediscovery, in cottonseed, of a toxic phenol-like substance, gossypol. This was obtained from the seed, where it occurs in relatively large amounts. It was later obtained, also, from the meal together with another form, D-gossypol, which differed in solubility.[2]

Withers and Carruth fed gossypol to rats, rabbits, pigs and chickens with varying effects.

The effect or toxicity of the gossypol added to the food or of the cotton seed meal itself was based upon growth and well-being of the animal. Feeding experiments were carried on for some time. When no untoward results, or at most only slight effects, were obtained, this was spoken of as a tolerance to gossypol. This occurs with the albino rat, likewise with chickens.

Later Schwartze[3] carried on extensive investigations on the distribution and extent of gossypol in various cottonseeds over the cotton belt. This investigator also studied the toxicity and pharmacology of gossypol on rats, rabbits, mice and guinea pigs. His measuring stick of the severity of the toxic effects on rats (with which most of the pharmacological work was done) was loss of appetite and loss of weight.[4] It was found that the threshold value of toxicity for the rat is 67.5 mgs. per kilogram weight. The minimum lethal dose is 30 to 50 mgs. per kilo when dissolved in oil and injected intraperitoneally or intravenously.

Shortly after this Jones and Waterman[5] showed that gossypol added to a pepsin-trypsin digestion in vitro affected the rate of digestion by retarding the enzymatic action. They fed a low concentration of gossypol containing approximately one-fifth as much gossypol as the amount contained in the daily consumption of cottonseed meal per head of cattle at our station. They obtained marked inhibition, both of digestion of the cotton seed globulin and of the casein to which the one per cent was added. They advance this inhibitory effect of gossypol upon digestion as a tentative explanation for the low coefficient of digestion (83 per cent) of cottonseed meal with animals.

The work reviewed above and that of others lead to the conclusion or belief that the toxic prin-

[1] The numbers refer to references at the conclusion of the article.

* Published with the approval of the Director of the North Carolina Experiment Station as paper number 22 of The Journal Series.

Presented at the 73rd Meeting of the American Chemical Society, Richmond, Va., April 14, 1927.

†*N. C. Agricultural Experiment Station.*

ciple in cotton seed was an explanation of the injurious results which were obtained when large amounts of cotton seed meal were fed. It should be noted that most of this work was done by feeding the seed or the gossypol isolated from the seed.

Another series of investigations on the nutritive value of cotton seed meal gave somewhat different results and interpretations. These will be reviewed briefly.

Rommel and Veddar[6] in 1915 published preliminary results of feeding cottonseed meal to pigs. Veddar had studied the effects of Beri beri in the Philippines and noted the marked similarity of symptoms. His conclusion was that the symptoms produced by feeding cotton seed meal to rapidly growing pigs was probably due to a deficiency of Vitamin B in the ration.

In 1916, Wells and Ewing[7] of the Georgia Station, feeding cottonseed meal to steers, produced certain effects from which they concluded that the problem resolved itself into one of an incomplete food.

Richardson and Green,[8] of the University of Texas in 1917, published results of extensive studies on the nutritive value of cottonseed meal. They were unable to demonstrate any effect of toxicity on the white rat. Upon the diets fed their rats reproduced several generations of young. Only when gossypol itself was added to the ration were any effects observed.

Recently in 1924, McGowan and Crichton[9] in Aberdeen, Scotland, have obtained injurious results when feeding cottonseed cake to young pigs. They corrected these results by adding iron oxide to the ration. They were able to simulate the symptoms by substituting peanut meal without the iron oxide. This produced similar lesions of the kidney. These investigators think that Withers and Carruth's results are due to deficiency. Their results, based upon a series of investigations, cause them to reject Withers and Carruth's interpretation that it is the toxic effect of D-gossypol[10] which produces injurious results in pigs.

From the above studies on the nutritive value of cotton seed meal were obtained different results from those obtained when isolated gossypol was added directly to the ration. In most of these experiments the amount of gossypol or D-gossypol, present in the cotton seed meal which was used, is not known. Some light is thrown upon these conflicting results by a recent publication—Studies in Gossypol: The Gossypol and D-gossypol content of some North Carolina Meals.[11] Of the 40 meals examined, it was found that 75 per cent of the gossypol in the seed is destroyed in the process of manufacture and that the amount of gossypol left in the meal is quite different from that found by Schwartze in the seed. Using the tolerance or threshold value of gossypol in the rat, the conclusion was reached that when cotton seed meal consituted 50 per cent of a balanced ration, that of the 40 meals examined the gossypol in only five of them (12½ per cent) would produce any toxic effect.

If there is a threshold value of toxicity, such an explanation may, in some measure, explain why cottonseed meal can at times be fed to farm animals in considerable amounts without apparent injury.

In 1926, Schwartze,[12] at The Tulsa Meeting of the American

Chemical Society, reviewed his previous work and attempted to apply his results, which were obtained from the analysis of the seed, to that of the meal. It is probable that this may not hold in view of the relatively great differences in the amount of gossypol in the seed compared to that in the meal. Bearing upon this point are several other facts. Schwartze found that the seed grown in the Southeastern States contained the greatest amount of gossypol and thus such meals themselves should contain a larger amount of the gossypol if this has not been destroyed in the process of manufacture. The amount of gossypol that meals contain in other sections of the Cotton Belt has not been determined. In this State 75 per cent of the gossypol in the seed is changed in the manufacture of meal to a less soluble form. Of the meals analyzed for gossypol, very few would produce any gossypol symptoms as shown by retardation of growth of the rat when ordinary or inconsiderable amounts constituted the ration (based upon the tolerance of the rat to gossypol). If, however, this substance cumulates in the body and is not eliminated as rapidly as ingested, then the effects of the smaller amounts of gossypol in the meal would depend upon the amount and length of time that the meal was fed.

Reference has been made above to the great reduction in the amount of gossypol in meal made from seed high in gossypol. It is not known what amount of gossypol remains in the meals made from seed relatively low in gossypol. Schwartze found that the different lots of seed varied greatly in the amount of gossypol which they contained. This substance would perhaps also vary still more in such meals after being subjected to heat and moisture in the process of oil extraction.

A changed form of gossypol, D-gossypol, is present in the meal, which in the hands of Withers and Carruth[13] was considered to be "of very slight toxicity for rats" or that it constituted a "far less toxic material." The toxicity of this substance has not been experimentally determined by feeding tests or by other means.

That gossypol itself is toxic there can no longer be any doubt. It causes at least two syndromes of symptoms, digestive and respirotory disturbances.

The quantity necessary to produce toxic or retardation-of-growth effects, with the exception of the rat, rabbit and cat, is not known. This applies to farm animals where the per cent of gossypol present in the meals fed, has not been estimated. Schwartze found 45 to 50 mgs. per kilo weight the largest survival dose for the rat when using intraperitoneal injections. This is 20.4 to 22.5 mgs. per pound live weight equivalent to 10.2 to 11.25 grams per 500 pounds (the weight of a heifer or steer).

The same investigator found 67.5 mgs. of gossypol per 100 gms. of food the threshold value of toxicity for the rat. These amounts are quite high when compared with the amounts ingested in the heavy feeding of cotton seed meal to dairy cattle at our station which are 45.3 mgs. gossypol per 100 pounds live weight.* (Approximate average of 10 head, or 0.3893 gram gossypol consumed per head per day.)

Recently we have fed 12.43 per cent cotton seed meal in the ration to growing swine with an average intake estimated at 35.3 mgs. gos-

* Soft Pork Studies by E. H. Hostetler and J. O. Halverson.

sypol* per day extending over 70 days to marketable weight with no apparent injury or arrest of gain in weight.

From this it appears that the amount of gossypol ingested in the meal by swine and dairy cattle is considerably less than the amounts experimentally found necessary to produce toxic effects on small laboratory animals. Gossypol is absorbed slowly when fed by mouth and is then apparently of a low degree of toxicity.

Icie G. Macy and Julia Outhouse[14] have recently summarized the effect of feeding cottonseed meal upon farm and small animals as follows:

Calves of 8 to 12 months are very susceptible.

Lactating cows are relatively immune.

Swine are particularly susceptible to cottonseed poisoning.

Guinea pigs and rabbits are affected.

Dogs show no effects in 120 days.

Sheep show no apparent symptoms.

The rat is more resistant.

The statements above as to calves and swine need, perhaps, in view of later work, some qualifications.

The results given in the literature on Gossypol Poisoning of Animals are chaotic.

One set of investigators fed seed comparatively high in gossypol and "isolated gossypol" in rather large amounts, in which the gossypol was injected intraveneously or added to the ration. In another series of experiments cottonseed meal comparatively low in gossypol was used in which the actual amounts present have not been determined. Doubtless the order of magnitude of gossypol ingested was not the same. The experimental work in feeding cottonseed meal gave results which might as readily be interpreted as being due to a deficiency as to toxicity. It is not improbable that rations containing large amounts of cottonseed meal may have been nutritively deficient in some respects where the animals were closely confined for long experimental periods. Factors such as these may have produced different results.

For farm animals the threshold value of toxicity of the gossypol present in cottonseed meal is not known. Apparently this is somewhat high, relatively speaking, thus enabling considerable cottonseed meal to be fed. Also the relatively low amount of gossypol left in the meal makes it possible to feed more meal with impunity. The D-gossypol in the meal seems to be held in a "bound" state and together with the gossypol does not appear to be actively toxic for farm animals in the sense of causing sudden deaths after short periods of feeding. Usually sudden deaths occur after somewhat longer periods of feeding or are preceded by swollen limbs, "fits" and a gradual decline and loss in weight. It is not known whether these two substances in cottonseed meal as ordinarily fed, exert a low order of toxicity on farm animals. Cattle and young stock fed a ration of which cottonseed meal constitutes a large part, get along fairly well for a considerable period of feeding.

In this problem are involved other factors among which are the condition of the animal and nutritive deficiencies in the ration. These deficiencies in the ration may be those due to the cottonseed meal itself and which are usually not

* Approximate figures obtained from the projects "Studies on Cottonseed Meal Feeding to Dairy Cattle" by R. S. Curtis, J. O. Halverson, and C. D. Grinnells.

properly supplemented in practical feeding of animals on farms.

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Referee Applicants

The following have applied for Certificates as Referee Chemists of The American Oil Chemists' Society; (first publication):

W. J. Bramblett, Texas Testing Laboratories, Fort Worth, Texas.

Industrial Laboratories, by H. M. Bulbrook and F. C. Schilling, Fort Worth, Texas.

A. H. Preston, Southwestern Laboratories, San Antonio, Texas.

H. M. Shilstone, New Orleans, Louisiana.

J. H. Sorrels, Terrell's Laboratories, Fort Worth, Texas.

Correction

Under "Standardization of Lovibond Glasses, Report for January, 1928," OIL AND FAT INDUSTRIES, March, 1928, page 93, first column, last line under Section I, for "....glasses in questionable" read "....glasses is questionable"

DON'T FORGET

Be with us in N'Orleans
Roosevelt Hotel
May 14, 15, 1928

The American Oil Chemists' Society

Notes and Correspondence

Standardization of Lovibond Glasses

(Investigation in Cooperation with A. O. C. S.)

Report for February, 1928

(Names in parentheses after subtitles and at ends of paragraphs indicate individuals principally responsible for the work described)

1. *Adoption of a Working Standard 35-Y.*

Considerable effort has been made to find a single glass which might be taken as a perfect standard 35-Y to be used in testing other glasses by direct comparison with it in the Martens photometer. This search has not met with complete success. These glasses depart from ideal in hue as well as in saturation. A glass may grade 35-Y so far as saturation is concerned and still be reddish or greenish relative to the standard. It may, for example, be equivalent to 35-Y O. 1-R. Failing to find a perfect standard in *one* glass, we have sought to make a standard 35-Y of *two* glasses. By carefully noting the errors of several glasses and the effects of combining them, we have found such a combination suitable to serve as a standard 35-Y (scale of Priest and Gibson derived from B.S. 9940), in so far as saturation and hue are concerned. (The transmission is, of course, not correct, but this is of no consequence in the way we use the standard.) This combination consists of the following two glasses:—

35-Y B.S. 10289 C
1-Y B.S. Test 41960.

This combination was arrived at as follows:—(1) It was noted that the true yellow numeral for the first named glass was 34.03 but that it was *greenish*. (2) It was noted that the true yellow numeral for the second named glass was 1.025, but that it was *reddish*. (3) This suggested that the combination might approximate closely to 35 and be neither green nor red relative to the standard. (4) The yellow numeral and the dominant wave length for this combination were computed from the previously determined spectral transmission of these glasses. The combination was found, by this computation, to be equivalent to

35. 1-Y 0.00-R.

The departure of 0.1-Y from 35.0-Y is of no practical consequence as 0.1-Y is entirely inappreciable by direct comparison of colors at about 35-Y. The fact that the red error is zero is the important consideration.

This combination served a very useful purpose in the final tests made on the 35-Y glasses submitted last August by the A.O.C.S. (Priest and Gibson).

II. Continuation of Investigation of the 35-Y Glasses Submitted by A.O.C.S. in August, 1927.

Investigation of the Lovibond 35-Y glasses, mentioned in the report for January, has been continued as follows:—

(1) Complete spectral transmission data have been obtained on the following glasses: A.O.C.S. Nos. 71, 75, 76, 101, 103, 114, 135, 165. The complete data on these glasses were taken because the preliminary partial data indicated that

these glasses were close to true 35-Y, and it was desired to test them more accurately in the hope of finding a particularly accurate 35-Y. The required computations are only about half finished and the results will be reported next month. (Gibson & Brown)

(2) Determination of the dominant wave lengths by direct comparison with a working standard glass has been completed. (Priest & Riley)

(3) Direct comparison of colors in the Martens photometer has given results in accord with the numerals found by Priest's empiric formula for the yellow numeral on scale of Priest and Gibson, viz:—

$$\text{Numeral} = -37 \log_{10}\left\{T_{480}/T_{550}\right\}$$

(Cf. Report for January.) This conclusion was reached by testing the correlation between Judd's observations on relative saturation and the computed numerals. (Judd & Priest)

(4) The true value of each glass in *yellow and red* has been finally arrived at by computation supplemented by direct observation, the steps being as follows:—

(a) The yellow numeral was computed, by Priest's formula, from measured values of transmission for wave lengths 480 and 550 millimicrons. (Cf. Report for January.)

(b) Standard yellow glasses were applied to neutralize the difference between these computed values and 35; and the resulting combinations were viewed in the Martens photometer, using as working standard 35-Y, the combination mentioned under I above.

(c) Red standard glasses were selected by trial to produce a perfect match in hue, and it was noted that in nearly all cases a saturation match was obtained. (Judd & Priest)

(5) All glasses received in August, 1927, which had not been previously returned, were returned (by Mr. Priest) to Mr. Trevithick, in New York, February 23, 1928.

(6) Preparation of report on these glasses is in course.

III. Temperature Coefficient of Color of Lovibond 35-Y Glass.

As is well known the color of a glass is not entirely independent of its temperature. With this in mind it has seemed desirable to determine the magnitude of this effect. Accordingly, the spectral transmissions for 35-Y have been determined at 15° and 35° C, a range intended to cover all ordinary conditions of use. From these data, the corresponding changes in sunlight transmission, dominant wave length, purity and Lovibond yellow numeral have been computed.

The sunlight transmission decreases with increase in temperature while the dominant wave length and purity both increase. The decrease in transmission is only 0.00047 per degree centigrade and is therefore quite negligible for practical purposes.

The change in purity is found to be 0.00026 per degree centigrade and is also negligible for practical purposes, even for most extreme variations of laboratory temperatures (say 15° C departure from average). It is true that the corresponding change in Lovibond numeral on the yellow scale is 0.136 per degree centigrade. That is, a change of 10°C really changes the yellow numeral by 1.4. This, however, is an inappreciable amount in color.

The change in dominant wave

length is found to be 0.020 millimicron per degree centigrade. A change of 10°C will accordingly change the dominant wave length by 0.2 millimicron which is equivalent to 0.1-R on the Lovibond scale. In more specific terms:—If two 35-Y glasses were exactly matched at 20°C and one of them were raised to 30°C while the temperature of the other remained at 20°, it would be necessary to add 0.1 R to the one at 20° in order to restore the hue match. (Gibson)

We have as yet no information as to corresponding changes in red glasses nor in cotton seed oil. It is, however, proposed to determine similar temperature coefficients for the glass *combination* 35-Y 7.6-R and for oil of this color. If it is found that the colors of the oil and this combination of glasses vary *together* with temperature the changes will be of no practical interest in color grading oil. If, on the other hand, the dominant wave lengths of oil and glasses should change in opposite directions with temperature, or if one should change much more than the other for the same temperature change, it is apparent that due account of this effect will have to be taken in reporting the color of oil.

IV. Further Calibration of Bureau of Standards Set of Lovibond Yellow Glasses (B. S. 9940).

In order to complete the calibration of fractional yellow glasses (numerals less than unity) in the set B.S. 9940, measurements on the spectral transmissions of these glasses have been started. Measurements have been made on the following glasses (designated by maker's numerals as marked on the glasses) :—0.90, 0.80, 0.70, 0.60, 0.50. The data obtained so far are not complete nor final. (Gibson & Brown)

V. Sensibility to Change in Yellow at 35-Yellow on the Lovibond Scale.

Observations made in connection with the calibration of sixty-five 35-Y glasses enable us to make what is believed to be a fair preliminary estimate of the order of magnitude of the difference in yellow just perceptible with certainty (with brilliance equated). This estimate is one to three units. Although the experiment by the method of right and wrong answers has not yet been made, these data indicate that the smallest difference for which a good observer would answer correctly 10 times in 10 trials would lie between one and three units. (Judd)

VI. Approximate Empiric Equivalence of Small Differences in Red and Small Differences in Yellow at 35-Yellow on the Lovibond Scale.

The following observations as to substitution of red for yellow result from the experiments made in calibrating sixty-five 35-Y glasses. If two nominal 35-Y glasses do not match *exactly* it often happens that an approximate match can be made by trial by adding either *red or yellow* to one or the other. It is difficult to decide which to add or whether some of both should be added. The practical equivalence appears from experience to be that 0.1 R may be substituted for 5-Y. (Judd)

VII. Review and Reduction of Earlier Data on Bureau of Standards Lovibond Red Glasses (B.S. 9940).

The review and reduction of earlier data by Dr. Frehafer, as mentioned in the report for January, has been continued. The com-

putations were nearly finished by the end of February, but not in form for final discussion. (Gibson & Riley)

VIII. Further Data on Bureau of Standards Lovibond Red Glasses (B.S. 9940).

The spectral transmissions of the following glasses (designated by maker's marked numerals) in set B.S. 9940 have been determined:— 3.7 and 1.85. (Walker)

IX. Design of Apparatus for Artificial Daylight Illumination to be Used with the Martens Photometer in Grading Glasses and in Testing Color Sense.

In order that observation may be made under constant conditions apparatus is being designed to provide a constant artificial daylight illumination to be used with the Martens photometer. (Priest & Walker)

X. Progress on Routine Calibration of Red Glasses Submitted by A.O.C.S. (Walker)

1. Fourteen red glasses (A. O. C. S. Nos. 201-214 inclus.) received January 16, were graded, reported and returned. (Date of report, February 10. Date of shipment to Mr. Putland, February 10.)

2. Nineteen red glasses (A. O. C. S. Nos. 215, 217, 222, 223, 225, 226, 229, 232, 234, 236, 257, 258, 273, 275, 279, 281, 282, 285.) received January 30, have been graded, reported, and returned. (Date of report, February 10. Date of shipment to Mr. Putland, February 10.)

3. Ten red glasses (special form, 16 mm disks) received January 30, were graded by February 16. Issue of report and return of glasses were delayed because of delay in receiving information as to identification numbers to be assigned to these glasses. (The report dated March 1, was mailed March 3, and the glasses were shipped to Law and Co., March 2.)

4. Twenty-five red glasses (A. O. C. S. Nos. 218, 254, 255, 289, 294, 296, 302, 306, 307, 308, 309, 315, 320, 321, 330, 331, 341, 342, 346, 351, 352, 353, 356, 358, 359) were submitted February 17. The grading of these was nearly completed February 29.

XI. Report on Tests of Color Sense Made at Memphis, May, 1927.

This report (by Priest) was mailed to the Editor of OIL AND FAT INDUSTRIES December 29 for publication. It developed during February that some revision would be desirable before publication. Mr. Priest conferred with Mr. Trevithick and Mr. Lee in New York February 23, and February 27, relative to such revision. The report is now being revised and it is expected that it will be published in OIL AND FAT INDUSTRIES for March or April. An abstract of this report (under the title, "Perception of Slight Color Differences") was published in the "Tech. News Bulletin of the Bureau of Standards" for January, 1928.

XII. Reprints of Report by Judd and Walker on "A Study of 129 Lovibond Red Glasses with Respect to the Reliability of their Nominal Grades."

This report, first published in OIL AND FAT INDUSTRIES, January, 1928, has been separately reprinted. A limited number of these separate reprints are available for free distribution. Address:—

Colorimetry Section,
Bureau of Standards,
IRWIN G. PRIEST,
Chief, Colorimetry Section.

SAMPLE REPORT

Bureau of Standards and American Oil Chemists' Society

Investigation of the uniformity of Lovibond glasses in use in the U. S.

Report on Calibration of Red Glasses Submitted by the A. O. C. S.

Date submitted

Lovibond Numeral as submitted (engraved on the glass)

Method of calibration used to obtain correct numeral

Method used to measure transmission

The following "Correct Numerals" relate to grading by hue. They are given in terms of the additive standards established by Priest and Gibson in May, 1927.

The "Transmission Error" is $(T_a—T_n)/T_n$, where

T_a is sunlight transmission of a combination consisting of the test glass and a 35-Y glass;

T_n is sunlight transmission of a combination consisting of the same 35-Y glass and a normal red glass having the numeral assigned to the test glass in this report.

A. O. C. S. No.	Other Marks Engraved on the Glass	Date of Calibration	Correct Numeral	Transmission Error	Remarks
Mean					

The errors in the "Correct Numerals" given above are believed to be less than 0.1.

Report Sheet No. .....

Washington .....
(Date)

Certified by
Research Associate of the A. O. C. S.
at the Bureau of Standards.

Announcement 1928 Annual Meeting New Orleans, Louisiana May 14th and 15th, Roosevelt Hotel, Also Color Vision Tests.

To the Membership:—

This meeting is going to be chock full of things doing. If there is a possible chance of being present you should be there.

In the past year, the matter of color vision and ability of oil chemists to read colors of refined oil has assumed enormous proportions of importance.

So Mr. Priest, of the Bureau of Standards, will be on hand at the meeting purposely, to test the ability of our members to read colors correctly on oils. All referee chemists, and all others interested should apply for these tests immediately, in order that Mr. Priest will know how to divide his time and what other arrangements to make. In writing me for the appointment, please state what day, Sunday, Monday, Tuesday, or Wednesday—13th, 14th, 15th or 16th—you would prefer to be tested. Also, if possible, what time of day you would prefer to take the tests.

The results of these tests will be considered in passing on referee chemists' certificates for 1928-29. Make all applications to me at 705 Tchoupitoulas Street, New Orleans, and I will forward notice to Mr. Priest.

The local committee promises big things in the line of entertainment this year. Mr. Ed Williams, Chairman, has something up his sleeve that he is not telling about now.

Let's make this meeting the biggest in the Society's history.

We are surely going to try our best to make you feel at home while you are down in old New Orleans.

Contest in Olive-Oil Chemistry

The National Association of Olive Growers of Spain is sponsoring an international competition the purpose of which is to bring forth an accurate and simple means of determining when an olive oil is mixed with other oil or oils and also what the other oils are that are in the mixture, and to extend the existing knowledge of the chemistry of olive oil. The association offers 20,000 pesetas as the first prize and 5,000 as second prize. The value of the peseta has recently been about six to the dollar. If none of the competitors scores enough points to win the first prize, this prize will be divided into four prizes of 5,000 pesetas each, all in the same category. Chemists of the United States are invited to enter the competition. The judging committee will consist of representatives of the National Association of Olive Growers of Spain, the Federation of Exporters of Spanish Olive Oil, the Body of Customs Experts attached to the Central Laboratory, a professor of the faculty of sciences of the Central University, and an agricultural engineer. The last date upon which works may be presented to the committee is July 30. Detailed information in regard to the competition may be obtained from the Department of State, Washington, D. C., or persons interested may communicate directly with Sr. Fernando Silvela, agricultural attache of the Spanish Embassy, Fifteenth and Fuller Streets, N. W., Washington, D. C.

Fig. 1. *Testing the Ability to Perceive Small Differences in Lovibond Red*

The observer, looking into the Martens photometer, sees a circular field divided by a vertical diameter. Both halves of the field are illuminated by daylight transmitted by the milk glass, the two halves being illuminated respectively by different parts of the milk glass. The Lovibond combination 35*Y* 7.6*R*, *being placed permanently between the observer's eye and the photometer, determines the color of both halves of the field. The difference glass* (*e.g.* 0.1*R*, 0.2*R*, *or* 0.3*R*) *is inserted between the milk glass and the photometer by the conductor of the experiment so as to affect the color of one half only, which may be either the right or the left at the option of the conductor of the experiment. In any one set of answers the difference glass is actually placed on the right as many times as on the left, but in an order unknown to the observer. The observer has no means of knowing on which side it has been placed except by his ability to perceive hue differences. He matches brilliance* (*by turning the circle, C, which rotates the nicol prism of the Martens photometer*) *and indicates the side on which he believes the difference glass to be, by saying "right" or "left." The conductor keeps a score of correct and incorrect answers*

OIL CHEMISTS

Be Sure to have your
Color Vision Tested at the
NEW ORLEANS CONVENTION
May 13, 14, 15 or 16

Write President Trevithick or Secretary Helm **at once**
for your appointment with Mr. Priest.